



## Brain Tumor Stem Cells and New Therapeutic Options

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### Abstract

Glioblastoma multiforme is a type of aggressive malign brain tumor that is resistant to treatment. Standard treatment consists of surgical resection, radiotherapy and chemotherapy. Despite all effective treatment combinations, the average survival time is under 15 months. Thus, more effective treatment goals are needed. The terms glioblastoma multiforme stem cell, cancer stem cell and brain tumor stem cell are used interchangeably. These cells are responsible for resistance to both radiotherapy and chemotherapy, tumor recurrence and the heterogeneous tumor structure. In the near future, genetical, epigenetical and molecular factors concerning tumor stem cells, the microenvironmental atmosphere and the immune system response to these will be constituting the larger part of brain tumor treatment studies. In this compilation, we aimed at presenting current information about brain tumor stem cells as well as new treatment options.

### Introduction

Gliomas constitute 80% of primary brain tumors and originate from normal stromal (glial) cells. Those that originate from astrocytes which are stromal cells are called astrocytomas, those that originate from oligodendrocytes are called oligodendrogliomas and those that originate from ependymal cells are called ependimomas. World Health Organization (WHO 2016) classification system is set up based on cell architecture and immunological marker profiles in addition to this stromal origin [1,2]. According to this system, astrocytomas are divided into four subgroups (Grade I - II - III and IV). The most aggressive and most commonly seen type of glioma is glioblastoma, which is grade IV astrocytoma. This tumor is defined by wide vascular endothelial proliferation, necrosis, high cellular density and tumoral heterogeneity. Glioblastoma formation may be caused by lower grade astrocytomas skipping grades (secondary glioblastoma - isocitrate dehydrogenase (IDH) mutant type) but mostly shows up as 'de novo' (primary glioblastoma-IDH wild-type) [3]. The standard therapy consists of surgical resection, radiotherapy and chemotherapy (specifically temozolomide). Despite all effective treatment combinations, the average survival time is under 15 months [4]. Today, glial tumor stem cells are deemed responsible for the aggressive structure observed in glial tumors, resistance to treatment and tumor recurrence [5]. In this article, we aimed at presenting information on brain tumor stem cells and new treatment strategies.

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### Stem Cells

Cells that are called embryonic or mesenchymal and which contribute to hemostasis in certain tissues have been recognized for a long time now. These self-renewing cells are called stem cells today. They are non-specialized cells with a biology specific to themselves. They take part in tissue development, hemostasis and the organization between different cell types. They divide asymmetrically and this allows for self-renewal and longer periods of stability (self-renewal). They can be turned into specialized cells by certain stimuli (pluripotency). They also have physiological regulatory mechanisms for organisms (regulability). These three main properties (self-renewal, pluripotency, regulability) determine the 'normal' stem cells of organisms. By dividing regularly, these cells regenerate tissues and replace the cells that are advancing towards apoptosis on their normal course. Through paracrine and autocrine cytokines, they connect both to other tissue groups and to the immune system. This way they maintain organisms [4,6,7].

### Neural Stem Cells

The cells that were presupposed to be Neural Stem Cells (NSC) were identified for the first time in the subventricular area of the rat brain in 1992. In the following studies, neural stem cells have been shown in many parts of fetal and adult brain. This discovery has changed our views about brain physiology significantly. The most important one of these changes is the view that neurogenesis continues into the adult life. In the central nervous system, various NSC's and neural progenitor

groups are found, starting from early embryogenesis to the adult phase. Today, NSC's have been defined in all phases from the embryo to the adult organism. They are found in subventricular zones, the subgranular zone and the special niches on the dentate curve of the hippocampus (microenvironment). These niches are found around vascular structures. Some other cells, extracellular factors such as Epidermal Growth Factor (EGF) and basic fibroblast growth factor are also found in these special pouches. Paracrine factors secreted by endothelial cells are among the important structures within the niches which promote the survival and self-renewal of the stem cells. This organization ensures that stem cells are in a close relationship with endothelial and other vascular cells. This facilitates the communication among different cell types. Hypoxia is a very important factor for the continuation as well as the proliferation of NCS's within the niches. These cells that are pluripotent and capable of self-renewal can transform into all neuroepithelial (neural and glial) cells [2,5,6,8]. Nonetheless, in order to be categorized as neural stem cells they need to have certain cell markers. Among these, Nestin, CD133 and CD44 are the most commonly known ones. Nestin, a stoplasmic microfilament is associated with cell skeleton, cellular signalization, organogenesis and the organization of cellular metabolism. CD133 plays a role in the development, migration and differentiation of the cell. Although similar to CD133, CD34 has been shown mostly in haemopoietic cells. CD44 is a transmembrane molecule which takes part in the adhesion between the cells located on the surface of NCS's and extracellular matrix. Other commonly accepted markers are Musashi-1, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E and Nanog [9-11].

## Glial Tumor Stem Cells-Brain Tumor Stem Cells

Tumor stem cells have been defined in pediatric as well as adult brain tumors. Studies have concentrated on the medullablastoma in the pediatric population and on the glioblastoma in the adult brain [4]. In the molecular studies that have been conducted, the differences of childhood brain tumors in comparison with adulthood brain tumors in terms of genetics, epigenetics and protein profile have been set forth. Based on glioblastoma gene expression profiles, 3 transcriptomic subtypes have been defined as proneural, mesenchymal and classical. In addition, 6 epigenetical subgroups have been set forth based on DNA methylation analysis [12]. Tumor stem cell bears the CD133, CD49f, CD36, A2B5, CD44, L1CAM and EGFR (epidermal growth factor receptor) markers. Transcription factors such as BMI1, Olig2 and SOX2 have been defined in tumor subgroups. Additionally, it has been found that in adult brain tumors DNA methylation profile changes and causes alteration in protein expression. This molecular profile shows also age group-dependant differences in childhood. In addition to the markers mentioned above, MELK (Maternal Embryonic Leucine Zipper Kinase) and PSPH (Phosphoserine Phosphatase) expression have been observed in childhood [4,13] (Table 1). Today, the terms glial tumor stem cell, Glioma Stem Cell (GSC) and Brain Tumor Stem Cell (BTSC) are used interchangeably [5]. Glioma stem cells may appear either by the transformation of normal embryonic stem cells or the transformation of progenitor cells back to the stem cell level in an abnormal manner. The reasons causing this are demonstrated as changes in pH, hypoxia, paracrine and autocrine cytokines and accumulation of genetic mutations formed by microglial response over time [14,15]. Eventually, a heterogeneous tumoral tissue is formed of the stem cells which have undergone changes at different

stages of development. This heterogeneity covers phenotype and genotype. The term clone refers to the cell groups which have the same genetics as the cell they originate from. The heterogeneity in the phenotype as well as genotype of the cell is referred to as clonal heterogeneity. This heterogeneous tissue that we come across in imaging or during surgery is a result of clonal heterogeneity. Hypoxia is an important factor that activates BTSC's. The BTSC rate increases in hypoxic areas. An increase in CD133 and alkaline phosphatase (another stem cell marker) is seen. There are studies showing that these increases are associated with prognosis. Hypoxic conditions regulate the swing of many growth factors. Hipoksi ile indüklelenen factor-1(HIF-1) and Transforming Growth Factor Beta (TGF- $\beta$ ) which may be induced by hypoxia play an important role in relevant pathways [3,16]. HIF-1, is a transcription factor which functions as the main regulator of oxygen homeostasis. Hypoxic niche regulates the tumorigenic capacity by HIF1 alpha and HIF2 alpha which are formed through overexpression of the ZNF217 gene. It has been reported that HIF decreases in BTSC's restrict self-renewal, survival and tumor formation abilities. Additionally, HIF plays a role in the regulation of Vascular Endothelial Growth Factor (VEGF) signal and the sustenance of angiogenesis. Hypoxic environment triggers VEGF expression in addition to these. Production of VEGF in high levels by BTSC's may increase angiogenesis and tumor initiation capacities. The hypoxic environment that develops within glioblastoma also contributes to the formation of the special hypoxic niche in which BTSC's can exist. Niche-tumor BTSC and BTSC-niche interaction is a two-way interaction. And this has much to do with the interaction between the tumor and the microenvironment [6,17]. Another important factor associated with hypoxia is pretein phosphatase 2 A (PP2A). PP2A activity and the regulator cell cycle within human glioblastomas are generated by hypoxia. PP2A reduces the metabolic needs of hypoxic BTSC's and boosts vitality in tumor cells. It has been determined that survival rates are lower in those patients who have higher PP2A activity as compared to those with lower PP2A activity [18]. BTSC's are not passively resident within the niches. These cells play active roles in tumor vascularity. BTSC's produce high levels of pro-angiogenic factors such as vascular endothelial growth factor (VEGF). BTSC's also produce differentiated progenes which exhibit endothelial cell properties and contribute to cancer-specific vascular formation [6,19]. Long-term hypoxic environment not only stimulates the expansion of CD133+BTSC's but also reveals the reprogramming potential of glioma cells whose origin is unknown to BTSC-like phenotype. As the reduction in vascular sustenance may have limited impact upon these cells, the ability of BTSC's to expand under hypoxic conditions causes additional difficulties in anti-angiogenic treatment. Various anti-angiogenic treatments may reduce vascular sustenance and activate BTSC's, thereby causing cancer invasion and metastasis [3,6,17]. In pediatric age groups, studies concerning the impact of hypoxia on BTSC's are not as many as those concerning adult BTSC's. It has been shown in a study that hypoxia inhibits p53 activation as well as astroglial differentiation that may cause high-grade gliomas. It has also been shown in the same study that BMP (Bone Morphogenetic Protein) signal pathway suppresses mitotic activity under high oxygen concentration conditions and that this signal is activated under conditions of low oxygen concentration [16,20,21].

## Communication Paths within Brain Tumor Stem Cells

There are 12 signal pathways associated with the main genes which

**Table 1:** Brain Tumor Stem Cells (child and adult).

Tumor Type	Stem Cell Markers	Active Signaling Pathways	Epigenetic Alteration
Pediatric Type Brain Tumors Stem Cells	Nestin	MYCN	ATRX
	CD133	Wnt/ $\beta$ -Katenin	BMP1
	CD15	Sonic hedgehog	EZH2
	SOX2	NOTCH	H3K27M
	CD49f	PI3K/Akt/mTOR	BMI1
	CD140a		FOXG1
SOX2			
Adult Type Brain Tumors Stem Cells	CD133	NOTCH/integrin	SOX2,
	EGFR	PI3K/Akt and MAPK	FOXM1,
	L1CAM	TGF- $\beta$	FOXG1,
	CD44	Wnt/ $\beta$ -Katenin	NANOG,
	CD36	Sonic hedgehog	STAT3,
	A2B5	VEGFR	GLI1,
	CD49f	L1CAM-integrin $\alpha$ 6	ASCL1,
		FACT	ZFX,
		HIF2 $\alpha$	ZFH4,
			HOXA10,
		EZH2/BMI1	

PI3K = Phosphor Inositide 3-Kinase, TGF = Transforming Growth Factor; VEGFR = Vascular Endothelial Growth Factor Receptor, mTOR = Mechanistic Target of Rapamycin, L1CAM = L1 Cell Adhesion Molecule, FACT = Facilitates Chromosome Transcription, HIF = Hypoxia-Inducible Factor, MYCN = N-myc proto-onkogen.

govern normal cell activity, are in charge of survival and genome continuity functions and regulate nuclear and cellular functions. Among these, Notch, Hedgehog and Wnt signal pathways are influential in embryonic development and adult tissue homeostasis. When these three pathways are activated in ways that diverge from the normal activation, it leads to tumoral formation in cells with stem cell -like phenotype or to the differentiation of brain tumor stem cells [13,15].

a) Notch Signal Pathway: Notch receptors are activated by ligands on the surface of the neighbouring cell. The intracellular parts of the activated Notch receptors are proteolytically released by the gamma-secretase complex, translocated into the nucleus and they subsequently activate the transcription of Notch-responsible target genes. Peritumoral endothelial cells secrete nitric oxide. Nitric oxide causes Notch activation in glioma cells. There are reports stating that the tumorigenic potential of stem cells is reduced and the growth of tumoral tissue is stopped [22].

b) Wnt Signal Pathway: The proteins secreted by the Wnt family and the associated receptors play an important role in embryonic development. Beta-catenin is the most important protein of the Wnt family. Beta-catenin is necessary for neuronal progenitor cell differentiation. Activation of Wnt signal encourages neuronal dedifferentiation. Increases malignancy in CD133 positive glioblastoma cells (especially in hypoxic environments). It is seen that these influences are adjusted through the antagonism of the Notch signal which regulates normal cell cycle. The different roles played by the Wnt signal in the glioma demonstrate the genetic heterogeneity of the signal as well [23].

c) Hedgehog Signal Pathway: The signal proteins secreted in Hedgehog play a critical role in embryonic tissue development and postnatal tissue homeostasis. When Hedgehog pathway is activated

at a time other than the normal timing, many malignancies develop and stem and progenitor cells become activated. Cellular reactions of the Hedgehog signal are regulated through Patched-1(PTCH1) and Smoothed (SMO) which are transmembrane proteins. The Hedgehog component and gene target expression levels are higher in grade 2 and 3 gliomas than they are in grade 4 gliomas. Therefore, the clinical benefit of targeting this pathway may be increased through studies performed in hedgehog-responsive glioma subtypes [6].

### Brain Tumor Stem Cell and Treatment

Following radiotherapy, the percentage of CD133+ (BTSC marker) cells within malign gliomas notably increases. This percentage depends on the elimination of tumor cells due to radiation or their survival depending on radiation resistance. The ability of BTSC's to repair radiation related DNA damage is shown in glioma tumor cultures. In addition to DNA damage repairment mechanisms, other paths that are preferably activated within BTSC's are also able to protect these cells against radiation related toxicity [17,24]. On the other hand, ionized radiation activates the Notch pathway in BTSC's, Notch signalization provides protection against radiation through the Akt related mechanism. Akt is a serine/threonine-specific protein kinase and plays an important role in apoptosis, cell proliferation, transcription and cell migration. Interestingly, after tumor recurrence, the rate of CD 133+ cells is associated with long-term survival. More advanced studies suggest that a significant proportion of CD 133+ cells in recurrent GBM samples are normal neural stem cells with potential antineoplastic activity. Conventional chemotherapeutic agents are produced out of studies performed on the final state of cancer cell clones. With the contribution of our knowledge on molecular level, there are important clinical studies conducted concerning the pathways that are effected the most in brain tumors and the medications inhibiting these pathways. These

consist of a limited number of tyrosine kinase receptor inhibitors, antiangiogenic factor inhibitors such as PDGFR  $\alpha/\beta$ , VEGFR, EGFR, PI3K and mTOR as well as antiproliferative and proapoptotic agents [25]. These agents are insufficient as they do not have any influence on cancer stem cells that both reproduce themselves and produce new tumoral tissue in the background. BTSC's are resistant to conventional chemotherapeutic agents too. This is because of the distinctive signal pathways of BTSC's. There are studies which hold the high activity of DNA damage repairment mechanisms and increased expression of ABC (ATP-Binding cassette) which is a membrane carrier protein responsible for this. Providing terminal differential cell type formation (especially like neurons) by blocking the brain tumor stem cell's self-renewal activity and encouraging differentiation may be a useful method in glioblastoma treatment [4,6,17,26]. Another system which stem cells have is the 5-fluorocytosine/cytosine deaminase enzyme system. Formation of 5-FU by external factors causes the inhibition of DNA and RNA synthesis. This enzyme system may be used as an effective means for prodrug activation within the tumor mass, resulting in the increase of local 5-FU concentration without systemic toxicity. This way, active cells among brain tumor stem cells can be brought under control [27]. In parallel with the increase in our knowledge on molecular level, developments in nano-scale particle technology offer new treatment opportunities. Nanotechnological products targeting tumor stem cell signal pathways are now able to manage and control stem cell differentiation. Efforts for the synthesized nanotechnological products to be taken into the cell are currently being carried on [4,6,20]. Another method used against brain tumor stem cells is immunotherapy. Activation of immune system by using cancer stem cells or some parts of the cancer stem cells is the key feature of immunotherapy. It is known that T lymphocytes play an effective role against tumor. Presentation of BTSC's or certain parts of them to T cells renders the immune response more effective against BTSC's [28]. Stimulation of T cells by a vaccine consisting of stem cell parts taken from the patient himself/herself is called active immunotherapy. Passive immunotherapy works by applying T cells activated by ex vivo prepared stem cell tumor antigen to the patients [5,29]. Another finding that is important in the treatment is the reducing and annihilating influence of normal neuronal stem cells on cancer stem cells. Keeping in mind the deteriorating immune systems of patients with glioblastoma, local autologous stem cell applications may be clinically applied in BTSC's at the early stage for the purpose of immunotherapy [25,6,30-32].

## Conclusion

Despite the fact that there are still many authors who have doubts about brain tumor stem cells, studies concerning treatments targeting pathways and receptors used by brain tumor stem cells are being conducted at an increasing frequency. In the near future, genetical, epigenetical and molecular factors concerning tumor stem cells, the microenvironmental situation and the immune system response to these will be constituting the larger part of brain tumor treatment studies. In the meantime, individual treatment will come into prominence. However, it seems to us that neurosurgical interventions will always be valid thanks to their impacts such as the elimination of mass effect, reduction of total tumor volume and tissue sampling.

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